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Biochemistry

Human cDNA clones for an α subunit of G_i signal-transduction protein

(receptors/adenylate cyclase/GTP-binding proteins/brain/mRNA)

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ABSTRACT **Two cDNA clones were obtained from a λgt11 cDNA human brain library that correspond to α_i subunits of G signal-transduction proteins (where α_i subunits refer to the α subunits of G proteins that inhibit adenylate cyclase). The nucleotide sequence of human brain α_i is highly homologous to that of bovine brain α_i [Nukada, T., Tanabe, T., Takahashi, H., Noda, M., Haga, K., Haga, T., Ichiyama, A., Kangawa, K., Hiranaga, M., Matsuo, H. & Numa, S. (1986) *FEBS Lett.* 197, 305–310] and the predicted amino acid sequences are identical. However, human and bovine brain α_i cDNAs differ significantly from α_i cDNAs from human monocytes, rat glioma, and mouse macrophages in amino acid (88% homology) and nucleotide (71–75% homology) sequences. In addition, the nucleotide sequences of the 3′ untranslated regions of human and bovine brain α_i cDNAs differ markedly from the sequences of human monocyte, rat glioma, and mouse macrophage α_i cDNAs. These results suggest there are at least two classes of α_i mRNA.**

Guanine nucleotide-binding proteins (G proteins) couple receptors for extracellular signals to effectors such as adenylate cyclase (1) or cGMP phosphodiesterase (2). G proteins consist of three protein subunits, α, β, and γ. α Subunits bind and hydrolyze GTP (1, 2) and display specificity for receptors and effectors. Different proteins, G_s and G_i, mediate stimulation and inhibition, respectively, of adenylate cyclase (where α_s and α_i are the corresponding α subunits). G_s and one or more forms of G_i are assumed to be present in most mammalian cells (1), whereas the α_t-1 subunit of transducin is expressed only in retinal rods (3, 4) and α_t-2 is expressed only in cones (4). Similarly, α_o (a G protein of unknown function) is abundant in brain but not in most of the other tissues that have been examined (5, 6).

The nucleotide sequences of cDNA clones for bovine (7, 8), rat (ref. 9; R. Reed, personal communication), mouse (10), and human α_s (11, 12) have been reported. R. Reed and coworkers have cloned and sequenced three types of α_i cDNA from a rat olfactory epithelium λgt10 cDNA library (personal communication). Other α_i cDNAs from bovine brain (13), bovine pituitary (14), human monocyte (15), mouse macrophage (10), and rat C6 glioma (9) have been sequenced. In addition, the sequences of rat (9) and bovine (16) α_o and bovine α_t-1 (17–19) and α_t-2 (20) cDNAs have been reported. The amino acid sequence homologies of α subunits range from ≈40% (α_s vs. α_i) to ≈78% (α_t-1 vs. α_t-2).

In this report, the nucleotide sequence of a human brain α_i cDNA is described and is compared with sequences of human monocyte (15), bovine brain (13) and pituitary (14), rat C6 glioma (9), and mouse macrophage (10) α_i cDNAs. Two types of α_i can be distinguished that differ in 12% of the amino acid residues and possess markedly different 5′ and 3′ untranslated sequences that have been conserved during evolution.

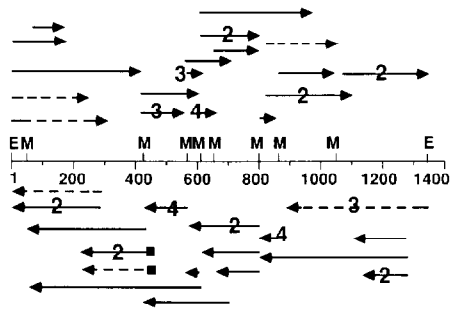

FIG. 1. Restriction fragments of BG-4 and BG21-2 α_i cDNAs were subcloned into M13mp18 and sequenced. Each arrow represents a subcloned DNA restriction fragment that was sequenced; arrow shafts composed of dashes represent nucleotide sequences from BG21-2 α_i cDNA; those with unbroken shafts represent sequences of BG-4 α_i cDNA. The numbers shown with some arrows represent the number of subclones of the same type that were sequenced. The number of nucleotide residues in human brain α_i cDNA is shown on the scale. E and M represent sites cleaved by *Eco*RI and *Mbo* I endonucleases, respectively.

METHODS

A λgt11 cDNA library was constructed by a modification of the method of Huynh *et al.* (21). Poly(A)$^+$ RNA was prepared from basal ganglia dissected from a 1-day-old human female brain and was used for cDNA synthesis. Duplex DNA >800 nucleotide pairs in length was ligated to λgt11 arms that had been dephosphorylated, and the DNA was packaged. The resulting library contains 10^6 cDNA recombinants; 90% of the phage contain DNA inserts.

Twenty-five thousand phage and 10^9 *Escherichia coli* Y1090 cells were plated per 150-mm Petri dish. Plates were incubated at 42°C for 2 hr and then at 38°C for 4 hr. Phage DNA was transferred to replicate nitrocellulose filters that were incubated in a solution containing 750 mM NaCl/75 mM sodium citrate, 1 mg of bovine serum albumin per ml, 1 mg of polyvinylpyrrolidone per ml, 1 mg of Ficoll per ml, 50 mM sodium phosphate (pH 6.8), 1 mM sodium pyrophosphate, 50 μg of yeast tRNA per ml, and 20% formamide for 16 hr at 42°C. Two probes, designed to hybridize to highly conserved regions of G-α subunit cDNAs (22), were synthesized. One probe, 43 nucleotide residues in length, consisted of 32 species of oligodeoxynucleotides, each containing six to eight

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Abbreviations: α_s and α_i, α subunits of guanine nucleotide-binding proteins (G proteins) that activate (G_s) or inhibit (G_i) adenylate cyclase; α_t-1, α subunit of transducin, a G protein of rod photoreceptor cells that activates cGMP phosphodiesterase; α_t-2, α subunit of transducin, a G protein of cone photoreceptor cells; α_o, α subunit of G_o, a G protein of unknown function.

deoxyinosine residues (5′ TCAT$^{T}_{C}$CTGCTT$^{C}_{I}$ACIATIGT$^{A}_{G}$-CT$^{T}_{C}$TT$^{C}_{I}$CCIGATTCICCIGCICC 3′).

The other probe consisted of a single species of oligodeoxynucleotide 50 nucleotide residues in length (5′ ACCTTGAAGATGATGGCGGTCACGTCCTCGAAGCCGTGGATCCACTTCTT 3′).

The 5′ terminal hydroxyl groups of the probes were labeled with ^{32}P from [^{32}P]ATP catalyzed by polynucleotide kinase. Each probe ($\approx 1.5 \times 10^6$ cpm/ml, 150 fmol/ml) was added to sets of four replicate 137-mm filters and incubated for 16 hr at 42°C. Each filter was washed three times in a solution containing 60 mM NaCl/6 mM sodium citrate and 0.1% $NaDodSO_4$ at 23°C for 20 min per wash, then washed once at 42°C in 60 mM NaCl/6 mM sodium citrate and 0.1% $NaDodSO_4$ for 3 min, and then subjected to autoradiography.

Phage from plaques yielding positive autoradiographic signals with both probes were cloned. DNA inserts were excised with *Eco*RI and subcloned into M13mp18, and partial nucleotide sequences of the subcloned single-stranded phage DNA inserts were determined by the dideoxynucleotide sequencing method (23). The complete nucleotide sequence of clone BG-4 was obtained by use of specific synthetic oligonucleotide primers and by sequencing BG-4 DNA fragments partially cleaved by *Mbo* I endonuclease and then subcloned.

Nucleotide or amino acid residues were aligned by using the NUCALN or PRTALN algorithms of Wilbur and Lipman (24). All amino acid residue alignments were performed with a K-tuple size of 1, window size of 20, and a gap penalty of 1. Alignments of nucleotide residues in the coding regions were performed with a K-tuple size of 3, window size of 20, and a gap penalty of 7; for 3′ and 5′ untranslated region alignments a gap penalty of 1 was used.

RNA for transfer blots was prepared from adult male human cerebral cortex and liver (25). Poly(A)$^+$ RNA was

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Ser Ala Glu Asp Lys Ala Ala Val Glu Arg Ser Lys Met Ile Asp Arg Asn Leu Arg Glu Asp Gly Glu Lys Ala Ala Arg Glu Val Lys   30
AGC GCC GAG GAC AAG GCG GCG GTG GAG CGG AGT AAG ATG ATC GAC CGC AAC CTC CGT GAG GAC GGC GAG AAG GCG GCG CGC GAG GTC AAG   90
                        CC       C TC                  AAG      G  G          A                  G       G

Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu Ala Gly Tyr Ser Glu Glu Glu   60
CTG CTG CTG CTC GGT GCT GGT GAA TCT GGT AAA AGT ACA ATT GTG AAG CAG ATG AAA ATT ATC CAT GAA GCT GGT TAT TCA GAA GAG GAG  180
T          T G          G  G  A  G  G  C  C  C  C              G  C       C  G  A   C  C  C  G       A

Cys Lys Gln Tyr Lys Ala Val Val Tyr Ser Asn Thr Ile Gln Ser Ile Ile Ala Ile Ile Arg Ala Met Gly Arg Leu Lys Ile Asp Phe   90
TGT AAA CAA TAC AAA GCT GTG GTC TAC AGT AAC ACC ATC CAG TCA ATT ATT GCT ATC ATT AGG GCT ATG GGG AGG TTG AAG ATA GAC TTT  270
  C CGG   G     CGG   G   T          C                    C  C  G  C  T G C  AA  C       A  AC C   C     C

Gly Asp Ser Ala Arg Ala Asp Asp Ala Arg Gln Leu Phe Val Leu Ala Gly Ala Ala Glu Glu --- Gly Phe Met Thr Ala Glu Leu Ala  119
GGT GAC TCA GCC CGG GCG GAT GAT GCA CGC CAA CTC TTT GTG CTA GCT GGA GCT GCT GAA GAA --- GGC TTT ATG ACT GCA GAA CTT GCT  357
 CC     C C T   A A       C   C   C A G   G   A      CA   G T C T C A C   C   G   G CAA     G G C C C    AT   C   G TCC

Gly Val Ile Lys Arg Leu Trp Lys Asp Ser Gly Val Gln Ala Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala Ala Tyr  149
GGA GTT ATA AAG AGA TTG TGG AAA GAT AGT GGT GTA CAA GCC TGT TTC AAC AGA TCC CGA GAG TAC CAG CTT AAT GAT TCT GCA GCA TAC  447
  C   C   C CG    G C C     GCT   C CA       G   G       C   T GG  C C   A A G   A           C   C   C   A   T   C

Tyr Leu Asn Asp Leu Asp Arg Ile Ala Gln Pro Asn Tyr Ile Pro Thr Gln Gln Asp Val Leu Arg Thr Arg Val Lys Thr Thr Gly Ile  179
TAT TTG AAT GAC TTG GAC AGA ATA GCT CAA CCA AAT TAC ATC CCG ACT CAA CAA GAT GTT CTC AGA ACT AGA GTG AAA ACT ACA GGA ATT  537
  C C     C     C    G C T   T   A   G AGT GAC          C   A   G          G   A C G   C C C    A   G   C   G   G   C

Val Glu Thr His Phe Thr Phe Lys Asp Leu His Phe Lys Met Phe Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys  209
GTT GAA ACC CAT TTT ACT TTC AAA GAT CTT CAT TTT AAA ATG TTT GAT GTG GGA GGT CAG AGA TCT GAG CGG AAG AAG TGG ATT CAT TGC  627
  G   G   A   C   C   C       G   C   A   C   C   G                   T         C G                             C   C

Phe Glu Gly Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Asp Tyr Asp Leu Val Leu Ala Glu Asp Glu Glu Met Asn Arg Met His  239
TTC GAA GGA GTG ACG GCG ATC ATC TTC TGT GTA GCA CTG AGT GAC TAC GAC CTG GTT CTA GCT GAA GAT GAA GAA ATG AAC CGA ATG CAT  717
  T   G   C   C   A   C                 C       C T    C   C   T    T   G          G   C   G   G          C

Glu Ser Met Lys Leu Phe Asp Ser Ile Cys Asn Asn Lys Trp Phe Thr Asp Thr Ser Ile Ile Leu Phe Leu Asn Lys Lys Asp Leu Phe  269
GAA AGC ATG AAA TTG TTT GAC AGC ATA TGT AAC AAC AAG TGG TTT ACA GAT ACA TCC ATT ATA CTT TTT CTA AAC AAG AAG GAT CTC TTT  807
  G           G C A   C   T       C   C                   C       C   G       C   C   C   C   C               C   G

Glu Glu Lys Ile Lys Lys Ser Pro Leu Thr Ile Cys Tyr Pro Glu Tyr Ala Gly Ser Asn Thr Tyr Glu Glu Ala Ala Ala Tyr Ile Gln  299
GAA GAA AAA ATC AAA AAG AGC CCC CTC ACT ATA TGC TAT CCA GAA TAT GCA GGA TCA AAC ACA TAT GAA GAG GCA GCT GCA TAT ATT CAA  897
  G   G   G       C C C   T       G   C   C      TC   T   G   C A    G G C      A       T           C AGC   C   C   G

Cys Gln Phe Glu Asp Leu Asn Lys Arg Lys Asp Thr Lys Glu Ile Tyr Thr His Phe Thr Cys Ala Thr Asp Thr Lys Asn Val Gln Phe  329
TGT CAG TTT GAA GAC CTC AAT AAA AGA AAG GAC ACA AAG GAA ATA TAC ACC CAC TTC ACA TGT GCC ACA GAT ACT AAG AAT GTG CAG TTT  987
A   A         G       G       G C C   A       C       G   C       G           G   C       C   C   C       C           C

Val Phe Asp Ala Val Thr Asp Val Ile Ile Lys Asn Asn Leu Lys Asp Cys Gly Leu Phe term                                    349
GTT TTT GAT GCT GTA ACA GAT GTC ATC ATA AAA AAT AAT CTA AAA GAT TGT GGT CTC TTT TAA GTTTTGCAGTCCATGGTAAAATGCATTTTCAAACC 1085
  G       C   C   C   C                 C   G   C   C   G   G   C   C   C       C  G  GGGGCAGCGGGGCCTGGCGGGATGGGCCACCGCCG

AAATGAGTACTTATATATGGATCTCTGTAGACTAGAGTCTTGCAGCAACACAGAATGTAATATAAGGCAAATGCATCTGGGACTTGACCAAAGTTGTTCTGTTTTGTTTTTTTAACTGA 1204
GAATTTGTACCCCCCAACCCCTGAGGAAGATGGGGGCAAGAAGATCACGCTCCCCGCCTGTTCCCCCGCCGCTTTTCTCCTCTTTCCTCTCTTTGTTCTCAGCTCCCCCTGTCCCCTCA

AAGTAACAGAAGGACCTTTCTTAAATGTGACAGATGGTCCTGCAGTTGAAACTGAAGGACAGTGTTAAAGCTGGGCTCTAGTATATTGATGATTTCTGCATAAGTGTAAATATGCAAAT 1323
GCTCCAAACGTAGGGGAGGGGTTCGCACAGGCCTCCCTGTTTGAAAGCCTGCCCTTGTCTGAGATGCTGGTAATGGCCATGGTACCCCCTTCTGGGCATCTGTTCTGGTTTTTAACCAT

GTATGTATACATGTATTTATG                                                                                                  1344
TGTCTTGTTCTGTGATGAGGG
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FIG. 2. Nucleotide sequence of human brain α_i cDNA and predicted amino acid sequence of α_i protein. On the third line of each set of lines are shown the nucleotide residues of human monocyte α_i cDNA (15) that differ from those of human brain α_i cDNA, except for the 3′ tail. Nucleotide residues 1–1276 correspond to BG-4 DNA. The regions of BG21-2 DNA that were sequenced correspond to residues 1–500 and 959–1344. The underlined nucleotides are the sites of hybridization of the 43-mer and 50-mer ^{32}P-labeled oligodeoxynucleotide probes. The first 10 nucleotide residues found in BG-4 DNA are GTGCCGAAAG, whereas the first 11 nucleotide residues found in BG21-2 DNA are TGCCGAAAGCG. We do not know whether these nucleotide residues are cloning artifacts, and therefore these residues are not shown.

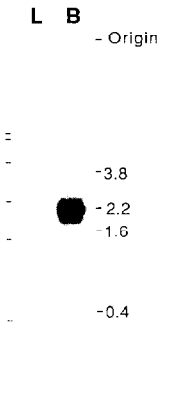

FIG. 3. Transfer blot analysis of poly(A)$^-$ RNA (20 μg per lane). Lane L, adult human liver RNA; and lane B, adult human brain RNA. The nitrocellulose filter was incubated with a [^{32}P]-RNA probe corresponding to the minus strand of 3′ untranslated region of BG21-2 α_i cDNA (nucleotide residues 1062–1344). The dash marks on the left indicate the chain lengths of the RNA markers used: 9.49, 7.46, 4.40, 2.37, 1.35, and 0.33 kilobases; on the right, the chain lengths of the radioactive brain RNA bands are shown.

isolated by oligo(dT)-cellulose column chromatography (26), fractionated by formaldehyde/agarose gel electrophoresis, and then blotted onto BA85 nitrocellulose membranes (Schleicher & Schuell). A probe specific for human brain α_i mRNA corresponding to BG-4 or BG21-2 α_i cDNA was obtained as follows: human brain α_i cDNA was subcloned into the *Eco*RI site of pGEM-blue 3 (Promega Biotec, Madison, WI). The recombinant replicative form DNA was converted to linear DNA by incubation with *Sca* I endonuclease; the cleavage site is 43 nucleotide residues past the termination codon in the 3′ untranslated region of α_i cDNA. The synthesis of a [^{32}P]RNA transcript complementary to 251 nucleotide residues in the 3′ untranslated region of human brain α_i was catalyzed by SP6 RNA polymerase (27). The nitrocellulose filters were prehybridized for 8 hr at 57°C in a solution containing 750 mM NaCl/75 mM sodium citrate, 5 mM sodium phosphate (pH 6.5), 1 mM EDTA, 0.5 mg of bovine serum albumin per ml, 0.5 mg of Ficoll per ml, 0.5 mg of polyvinylpyrrolidone per ml, 0.1% NaDodSO$_4$, 200 μg of yeast tRNA per ml, and 50% formamide. The [^{32}P]RNA α_i-specific probe was added (1×10^6 cpm/ml, 4 fmol/ml) and the reaction mixture was incubated 18 hr at 57°C. The filter was washed three times in a solution containing 15 mM NaCl/1.5 mM sodium citrate and 0.1% NaDodSO$_4$ at 55°C, then washed two times at 65°C for 20 min each wash, and then subjected to autoradiography for 18 hr with an intensifying screen.

RESULTS AND DISCUSSION

Sequence of Human Brain α_i cDNA. A λgt11 cDNA library prepared from total cellular poly(A)$^+$ RNA from 1-day-old human basal ganglia was screened with two ^{32}P-labeled oligodeoxynucleotide probes complementary to highly conserved regions of α subunits of G proteins (22). Fourteen of the 575,000 cDNA recombinants screened gave positive signals with both probes. Part of the nucleotide sequence of each positive clone was determined, which led to the identification of 2 α_i cDNA clones, BG-4 and BG-21-2, and 11 α_s cDNA clones (11). Both strands of human brain BG-4 α_i cDNA and part of BG21-2 cDNA were sequenced (Fig. 1). Most regions of BG-21-2 DNA that were sequenced proved to be identical to the corresponding sequence of BG-4 (with one exception noted in the legend to Fig. 2); however, the chain length of BG21-2 was longer than BG-4. The nucleotide sequence of BG-4 human brain α_i cDNA (residues 1–1266) and the additional BG-21-2 sequence (residues 1267–1344) are shown in Fig. 2 and are compared with the recently reported nucleotide sequence of human monocyte α_i cDNA (15). The first nucleotide of BG-4 corresponds to the 16th residue in the coding region of human monocyte α_i. Nucleotide residues 1–1047 comprise an open reading frame coding for 349 amino acid residues followed by a termination codon and 294 additional 3′ untranslated nucleotide residues. Two-hundred and seventy-seven of the nucleotide residues scattered throughout the coding portion of human brain α_i cDNA differ from those of human monocyte α_i cDNA (27%) (15). However, 221 of the nucleotide substitutions are silent mutations and 56 result in the replacement of 42 of the 349 amino acid residues compared (12%). Little or no homology was found in the nucleotide sequences of the 3′ untranslated regions of human brain and monocyte α_i cDNAs (67% of the residues differ). These results show that the nucleotide sequences of human brain and monocyte α_i cDNAs differ substantially and suggest that human brain and monocyte α_i mRNAs are transcribed from different genes. These results agree well with the findings of R. Reed and his colleagues that rat olfactory epithelium contains three types of α_i (personal communication).

Transfer Blot Analysis of Human α_i mRNA. A [^{32}P]RNA probe complementary to nucleotide residues 1062–1344 in the 3′ untranslated region of BG-21-2 human brain α_i cDNA was incubated with human liver and brain poly(A)$^+$ RNA that had been fractionated by gel electrophoresis and transferred to a nitrocellulose filter (Fig. 3). The [^{32}P]RNA probe was expected to hybridize with human brain α_i mRNA corresponding to BG-4 or BG21-2 cDNA but not to other species of α-mRNA. Two faint, diffuse bands of radioactive liver poly(A)$^+$ RNA were detected with chain lengths of 1.7 and 1.0 kb, and one major and three minor radioactive bands of brain poly(A)$^-$ RNA were found with chain lengths of 2.2, 3.8, 1.6, and 0.4 kilobases (kb), respectively. The 3.8-kb α_i poly(A)$^+$ RNA from human brain is similar in size to the 3.9-kb chain length reported for bovine brain α_i mRNA (13).

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H BRAIN            SAEDKAAVERSKMIDRNLREDGEKAAREVKLLLLGAGESGKSTIVKQMKIIHEAGYSEEECKQYK  (70)
B BRAIN        MGCTL·····                                                            70
H MONOCYTE     ····V·····A·····K·····D·····R··R                                      70
R C6 GLIOMA    ····V·····A·····K·····D·····R··R                                      70
M MACROPHAGE   ····V·····A·····K·····D·····R··R                                      70

HB  AVVYSNTIQSIIAIIRAMGRLKIDFGDSARADDARQLFVLAGAAEE-GFMTAELAGVIKRLWKDSGVQACFNRSREYQLN (149)
BB  ·····                                                                           149
HM  ··········M··VK···N·Q···A·PS·········A·SCT···Q·VLPDD·S···R···A·H·····G······    150
RG  ··········M··VK···N·Q···A·PQ·········A·SC····Q·MLPED·S···R···A·H·····G······    150
MM  ··········L··VKR··N·Q···A·PQ·········A·SC····Q·MLPED·S···R···A·H·····G······    150

HB  DSAAYYLNDLDRIAQPNYIPTQQDVLRTRVKTTGIVETHFTFKDLHFKMFDVGGQRSERKKWIHCFEGVTAIIFCVALSD (229)
BB  ·····                                                                           229
HM  ··········E····SD·····                                                          A 230
RG  ··········E····SD·····                                                          A 230
MM  ··········E····SD·····                                                          A 230

HB  YDLVLAEDEEMNRMHESMKLFDSICNNKWFTDTSIILFLNKKDLFEEKIKKSPLTICYPEYAGSNTYEEAAAYIQCQFED (309)
BB  ·····                                                                           309
HM  ·····TH·····F···T·A·K·D···S···SK···                                             310
RG  ·····TQ·····F···T·A·K·D···S···SK···                                             310
MM  ·····TQ·S····F···T·A·K·D···S···SK···                                            310

HB  LNKRKDTKEIYTHFTCATDTKNVQFVFDAVTDVIIKNNLKDCGLF (354)
BB  ·····                                         354
HM  ·····                                         355
RG  ·····                                         355
MM  ·····                                         355
```

FIG. 4. Amino acid sequence of α_i from human brain compared with α_i sequences from bovine brain (13), human monocytes (15), rat C6 glioma cells (9), and mouse macrophages (10). The letters represent the single-letter abbreviations for amino acids. The symbol · represents an amino acid residue that is identical to the residue shown for human brain α_i; – represents a gap.

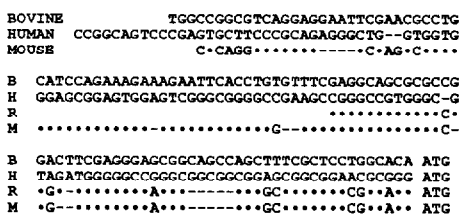

FIG. 5. Nucleotide residues in the 5′ untranslated regions of bovine brain α_i-1 cDNA (13) and human monocyte α_i-2 cDNA (15). The symbol · represents a nucleotide residue in rat (9) or mouse (10) α_i cDNA that is identical to the residue shown in human monocyte α_i cDNA. Rat and mouse nucleotide residues that differ from those of human macrophage cDNA are shown. ATG at the 3′ end of each sequence represents the initiation codon for methionyl-tRNA.

These results reveal tissue-specific differences in the expression of human α_i-1 mRNA.

Comparison of α_i Amino Acid Sequences. The predicted amino acid sequence of human brain α_i is compared in Fig. 4 with α_i amino acid sequences predicted from nucleotide sequences of cDNAs from bovine brain (13), human monocytes (15), rat C6 glioma cells (9), and mouse macrophages (10). The predicted amino acid sequence of human brain α_i is identical to that of bovine brain α_i (13) and differs in only 3 amino acid residues from bovine pituitary α_i (14) (not shown). In contrast, human and bovine brain α_i differ from human monocyte, rat C6 glioma cells, and mouse α_i in ≈12% of the amino acid residues. The amino acid sequences of α_i from human monocytes, rat, and mouse are closely related (99% homology) and contain a codon for an additional amino acid residue, Gln-117, which is not present in human brain, bovine brain, or bovine pituitary α_i cDNAs. These data reveal two types of α_i cDNA: α_i-1 from human brain, bovine brain (13), and bovine pituitary (14), and α_i-2 from human monocytes (15), rat C6 glioma cells (9), and mouse macrophages (10). Thirty-six of the 44 amino acid residues of α_i-1 and α_i-2 that differ are clustered in two regions: region A (amino acid residues 82–142) with 25 residues that differ and region B (amino acid residues 280–309) with 11 residues that differ. Furthermore, only 55% of the amino acid replacements are conservative replacements (28). Regions A and B contain the greatest diversity in amino acid sequence in the α family of proteins and may contain sites that determine the specificity of G-protein interactions with effectors and receptors, respectively (29).

The predicted secondary structures of α_i-1 and α_i-2 based on the parameters of Chou and Fasman (30) differ in interesting ways. Amino acid residues 118–124 of α_i-1 (the numbering system is that of bovine brain α_i-1 shown in Fig. 3) are predicted to form an α-helix that is not present in α_i-2. Conversely, amino acid residues 97–100 and 120–123 of human monocyte, rat, and mouse α_i-2 are predicted to form β-turns that are not present in human and bovine α_i-1 protein subunits. The differences in predicted secondary structures are located in a variable region of α proteins that is thought to interact with effector molecules.

The amino acid sequence of human α_i-1 is identical to that of bovine α_i-1, whereas the amino acid sequences of human, rat, and mouse α_i-2 differ from one another by 3–8 amino acid residues. These amino acid replacements may have resulted from relatively recent mutations during the last 8.5×10^7 years because human, bovine, and rodent precursors diverged from a common ancestor $\approx 8.5 \times 10^7$ years ago (33). However, the amino acid sequences of human and bovine α_i-1 differ from the sequences of human, rat, and mouse α_i-2 in 36 additional amino acid residues, and the mutations that resulted in these amino acid substitutions must have occurred $> 8.5 \times 10^7$ years ago. Such considerations lead us to speculate that α_i-1 and α_i-2 mRNA are transcribed from separate genes that originated by duplication of an ancestral α_i gene much more than 8.5×10^7 years ago and then diverged over a long period of time by accumulation of mutations. The differences between the amino acid sequences of α_i-1 and α_i-2 are likely to be functionally significant, since the differences apparently have been conserved during evolution. In some ways the relatedness of α_i-1 and α_i-2 resembles that of α_t-1 (17–19) and α_t-2 (4, 20), which exhibit 78% amino acid homology and interact with rhodopsin in retinal rods and opsin pigments in cones, respectively.

α_i Nucleotide Sequences. Comparison of α_i cDNA nucleotide sequences from different organisms (not shown here) provides additional evidence for two types of α_i. The nucleotide sequence of human brain α_i cDNA closely resembles that of bovine brain (13) and bovine pituitary (14) α_i cDNAs (94% homology); in addition, human monocyte (15), rat C6 glioma (9), and mouse macrophage (10) α_i-2 cDNA nucleotide sequences closely resemble one another (87–90% homology). However, α_i-1 nucleotide sequences differ substantially from those of α_i-2.

As shown in Fig. 5, the nucleotide sequence of the 5′ untranslated region of bovine brain α_i-1 differs markedly from the corresponding sequences of human monocyte, rat C6 glioma, and mouse macrophage α_i-2 cDNAs (33% homology). However, the 5′ untranslated nucleotide sequences of α_i-2 cDNAs from human monocytes, rat, and mouse closely resemble one another, which suggests that the α_i-2 5′ untranslated nucleotide sequences have been conserved during evolution.

Comparison of the initial nucleotide residues in the 3′ untranslated regions of α_i cDNAs (Fig. 6) shows that human and bovine brain (13) α_i cDNAs are closely related (92% homology) and that human monocyte and rat α_i cDNAs are related to one another (83% homology). However, little or no homology was detected between the 3′ untranslated regions of human brain and bovine brain α_i cDNAs compared to

FIG. 6. Nucleotide sequences at the beginning of the 3′ untranslated regions of human brain α_i-1 and human monocyte α_i-2 cDNAs (15). The nucleotide sequence of bovine brain α_i-1 cDNA (13) is compared with the sequence of human brain α_i-1 cDNA, whereas the nucleotide sequences of rat (9) and mouse (10) α_i-2 cDNAs are compared to the nucleotide sequence of human monocyte α_i-2 cDNA. TAA or TGA at the 5′ terminus represents termination codons for α_i cDNAs. The symbol · represents a nucleotide residue that is identical to the residue shown for human brain α_i-1 or human monocyte α_i-2 cDNA. Nucleotide residues that differ are shown.

```
HUMAN  TAAGAAGGGAACCCCCAAATTTAATTAAAGCCTTAAGCAC
BOVINE TAA··········T···G··········G·········G·
RAT    TAA·········G·············C·-···········
MOUSE  TAA·········A·············C·-···········

H  AATTAATTAAAAGTGAAACGTAATTGTACAAGCAGTTAATCACC
B  ··············A·G·TA··········C·····G·····
R  ··········G·········C···C···········G·····
M  ··········G·····························GG·····

H  CACCATAGGGCATGATTAACAAAGCAACCTTTCCCTT-CCC
B  ·····································T···
R  ···············C····CC·············TT·T
M  ···············C····CC···········T··TT··
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FIG. 7. Nucleotide sequences at the beginning of the 3′ untranslated regions of α_s cDNAs from human brain (11) and human liver (12). The human nucleotide sequence is compared with the sequences of α_s cDNAs from bovine brain (7) and bovine adrenal medulla (8), which are identical in this region, and with rat C6 glioma (9) and mouse macrophage (10) cDNAs. TAA at the 5′ terminus represents the termination codon for α_s cDNA. The symbol · represents a nucleotide residue that is identical to that shown for human α_s cDNA; nucleotide residues that differ are shown.

human monocyte, rat, and mouse α_i-2 cDNAs. The sequence of the first 25 nucleotide residues from the 3′ untranslated region of mouse α_i cDNA matches the initial 3′ untranslated sequences of human monocyte and rat α_i cDNAs (92–96% homology), but thereafter, the sequences are not related.

As shown in Fig. 7, the nucleotide sequences of the 3′ untranslated regions of human, rat, mouse, and bovine α_s cDNAs also are highly conserved (90–93% homology). However, the initial portion of the 3′ untranslated nucleotide sequence of human brain α_s cDNA (11, 12) is not related to the 3′ untranslated sequences of human α_i-1 or α_i-2, rat α_o (9), or bovine α_i-1 (17–19) or α_i-2 (20) cDNAs (not shown). The relatively high homologies in untranslated regions of α_i-1, α_i-2, or α_s mRNAs in different species suggest that the untranslated nucleotide sequences are functional and thus have been conserved during evolution.

The 3′-terminal untranslated region of bovine brain α_i-1 cDNA contains many repeats of (A+T)-rich sequences similar to the consensus sequences TTATTTAT (34) and TT(G/A)NNNTTTTTTT (35), which have been found in the 3′ untranslated regions of some species of mRNA and have been proposed to function as signals for rapid turnover of mRNA (36). The (A+T)-rich sequences are less frequent in α_o and α_s and have not been found in human, rat, or mouse α_i-2 cDNAs. Whether α_i-1 mRNA turns over more rapidly than α_i-2 mRNA remains to be determined.

The nucleotide sequences in the 3′ untranslated regions of β-actin, cardiac α-actin, c-*fos*, nerve growth factor, and creatine kinase mRNAs from different organisms also have been conserved during evolution (see ref. 37 for discussion). Different forms of actin and creatine kinase with conserved 3′ untranslated regions have been shown to be the products of separate genes that are expressed in different tissues and at different times during development.

Data from α cDNAs have revealed an unexpected diversity in α_i and α_s (7–18). Comparison of human brain and human monocyte (15) α_i cDNAs suggests that the two types of human α_i are transcribed from separate genes. The nucleotide sequences of α_i cDNAs reveal that α_i genes are subject to strong selective pressure in the coding region and the 5′ and 3′ untranslated regions. Comparison of amino acid sequences predicted from α_i cDNAs suggests that α_i-1 and α_i-2 proteins may differ in function as well as in tissue distribution and abundance.

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